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Next Meeting
Sydney, Australia
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ORAL PRESENTATIONS

Generation of Skin Substitutes for the Treatment of Burns

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The use of skin substitutes to facilitate wound closure has significantly improved the survival rate of patients with insufficient donor skin for autografts. These skin substitutes can be categorised into three classes: (1) cultured epidermal sheets or suspensions; (2) dermal replacements consisting of a biosynthetic matrix with/without fibroblasts, and (3) two-layered constructs comprising a combination of skin cells in a matrix. These substitutes are available commercially or are produced locally by hospital laboratories. The Skin Engineering Laboratory (SEL) of the Burns Unit, Royal Adelaide Hospital is one such laboratory and is the first laboratory in Australia to be licensed by the Therapeutic Goods Administration to produce cultured keratinocytes for clinical use. This Autologous Cultured Keratinocytes for Transplantation (ACKT) process is performed entirely in Class 350 cleanrooms in a facility with an established quality control system to ensure product quality and safety. ACKT has been used successfully in 14 major (>50% of total body surface area (TBSA) burns cases and provided sufficient numbers of cells to cover > 100% TBSA per patient.

The establishment of the cultured skin cell service is the first step towards the generation of wound healing and skin regenerative products by SEL. Our primary aim has always been to develop skin substitutes that closely approximate autologous skin. We report on the potential use of a novel family of polyurethanes, NovoSorb™, as the scaffold for skin substitutes. These Biodegradable Temporising Matrices (BTMs) are engineered to have the required mechanical properties of skin and demonstrate high biocompatibility with keratinocytes, dermal fibroblasts and microvascular endothelial cells in *vitro* and *in vivo* studies. Furthermore, preliminary studies show BTM reduced wound contraction and supported reepithelialisation when transplanted onto full thickness wounds in sheep. Cell infiltration studies and construction of skin substitutes using BTM are currently underway.

A New Role for Pericytes in Promoting Epidermal Regeneration

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Epidermal stem and progenitor cells of the skin interact with their stromal microenvironement to regulate epithelial tissue renewal and maintenance. However, the precise molecular and cellular components that form the microenvironment of keratinocyte stem cells and their progeny remain poorly defined. Recent studies in our laboratory have provided insight into the role of the extracellular matrix component laminin-10/11 in promoting skin tissue regeneration. In addition we identified a functionally relevant subset of human dermal cells identified by the antibody HDF-1, (HDF-1^{bri} cells), which secrete soluble factors that promote human skin regeneration. In this study we have sought to determine the identity, location and molecular profile of HDF-1^{bri} cells using microarray technology with the specific goal of identifying functional molecular regulators provided by these cells that may act to promote skin regeneration, while providing new markers for this functionally relevant subset of dermal cells. The gene expression profiling data reveal that HDF-1^{bri} cells preferentially express various mediators of epithelial-mesenchymal interactions at the m-RNA level, including growth factors, extracellular matrix proteins and signalling molecules including the Notch pathway recently implicated in stem cell regulation. Further, new cell surface markers that identify the HDF-1^{bri} cells have also been identified. These data provide the basis for the functional analyses of molecular regulators that promote the proliferative and tissue regenerative ability of keratinocyte stem cells and their progeny with the long-term aim of developing improved therapeutics for human skin replacement.

Flightless 1: A Novel Target for Improving Wound Repair

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The actin cytoskeleton is integrally involved in cellular processes including adhesion, migration and proliferation all essential processes for wound repair. Our studies investigating Flightless 1 (Flil), a highly conserved actin-remodelling protein, now reveal that Flil is an important regulator of wound repair whose manipulation may lead to enhanced wound outcomes. Using Flil deficient (+/−) and Flil transgenic mice we have investigated the effect of differential Flil gene expression on wound healing. Flil deficient (+/−) mice are characterised by improved wound healing and enhanced reepithelisation. In contrast, Flil over expressing mice have significantly impaired wound healing with reduced cellular proliferation and delayed epithelial migration. Although Flil was thought to be solely an intracellular protein we have new evidence showing that it is secreted in response to wounding. We have now generated and tested a panel of affinity purified mouse polyclonal antibodies raised against different parts of the Flil peptide and have shown that they can modulate Flil activity *in vitro*. Intradermal application of one of these antibodies (αFlil) to murine incisional wounds significantly enhances wound healing showing that modulation of Flil directly affects wound repair outcomes. We have further investigated how Flil may modulate wound repair by studying its effects on two growth factors known to be important in fibrosis and scar formation. TGF-β1 expression is decreased in Flil deficient wounds whilst TGF-β3 is enhanced. This ratio of low TGF-β1:high TGF-β3 could contribute to the enhanced healing observed in Flil deficient mice. Additionally, when Flil gene expression was knocked down by over 90%, using siRNA a significant decrease in Smad2 and 3 gene expression was observed yet inhibitory Smad7 was significantly elevated. We have also investigated the effect of Flil on the AP-1 proteins c-fos and c-jun and found that when Flil levels were reduced by siRNA for 30 minutes, both c-fos and c-jun mRNA were significantly decreased compared to controls. Our studies have shown that Flil is a potentially important mediator of wound repair via mechanisms involving cell migration and proliferation. Additionally, Flil may modulate TGF-β gene expression via regulation of AP-1 and signalling via the Smad pathway. Understanding these important processes will help to identify new targets for modulating Flil activity thereby leading to potential new mechanism-based wound and anti-scarring therapies.

Snail Transcription Factors Down-regulate Keratinocyte Differentiation Genes

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Background: The Snail family of zinc finger transcription repressors is known to induce an epithelial mesenchymal transition (EMT) in epithelial cells and are over-expressed in squamous cell carcinomas. A prominent target of Snail is E-cadherin, a cell-cell adhesion protein that is important in maintaining polarity and a differentiated epithelial phenotype. It has been reported that stable transfection of MDCK kidney epithelial cells with a Snail-expression vector is sufficient to confer tumorigenicity and invasiveness on them. However, recent reports also have shown that re-expression of E-cadherin from a Snail-insensitive promoter is not sufficient to reverse the EMT in Snail-expressing cells. The full complement of genes targeted by Snail in keratinocytes are not known.

Method: In order to identify other Snail target genes that are important to EMT, we have developed a tamoxifen-inducible Snail model based on the well characterized MDCK cell line. A chimeric gene was made by fusing human Snail-1 at its C-terminus to ERT2, a mutant form of the human estrogen receptor that is inducible by tamoxifen. This inducible model was validated as accurately recapitulating Snail up-regulation in epithelial cells using MDCK cells, and then applied to keratinocytes of both head & neck and skin origin to identify target genes in those cells, using cDNA microarrays.

Results: As well as epithelial-specific cell-cell adhesion protein-encoding genes, several genes with critical roles in terminal differentiation of keratinocytes were demonstrated to be down regulated by Snail.

Conclusion: The genes identified suggest that Snail may contribute to tumour pathogenesis by preventing terminal differentiation, as well as increasing invasion of extracellular matrix.

Visualising Dendritic Cell Responses in the Skin

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The skin harbours specialised subsets of innate immune cells, including epidermal Langerhans cells (LC), dermal dendritic cells (DDC) and dermal macrophages. These cells are believed to regulate cutaneous immuno-surveillance by virtue of detecting invading microbes, and transporting them to draining lymph nodes. Despite these important activities, we have little understanding of the spatio-temporal orchestration of cutaneous innate immunity. We made use of a newly-developed intravital two-photon microscopy model in mouse skin in order to visualise the behaviour of LC and DDC at the single cell level in real time within their natural microenvironment. To this end, we employed transgenic mice in which the enhanced yellow fluorescent protein (YFP) is driven under the control of the CD11c promoter, In the steady-state, LC were found to be immobile, with only occasional extensions and retractions of their dendrites. In stark contrast, DDC were actively crawling through the dermal interstitial space, suggesting active immuno-surveillance at this site. Upon challenge of mice with endotoxin, DDC underwent remarkable shape change as evidenced by their elongation, and decreased motility. These alterations persisted for approximately three hours. After six hours, DDC resumed migratory behaviour at a level comparable to untreated mice. In order to further analyse their immunosurveillance function, fluorescently-tagged *Leismania major* parasites were injected intradermally. Interestingly, we found that some of the parasites moved freely through the extravascular space. However, we also observed vacuolisation of YFP+ DDC shortly after parasite injection, indicating active infection of these cells. Taken together, we have defined a microanatomical localisation specific migratory behaviour of DC in the skin. In the case of DDC, this enables the cells to rapidly encounter intruding pathogens, which represents the earliest step of cellular innate immune responses.

Differences in MHC Expression Between Melanocytes of the Hair Follicle and Epidermis

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The anagen hair bulb is one of the few sites of immune privilege (IP) in mammalian tissues[1]. A collapse in hair follicle IP, with increased expression of major histocompatibility complex (MHC) class Ia and MHC class II, is thought to be the mechanism behind alopecia areata (AA)[2] resulting in patches or widespread areas of alopecia. In contrast, the autoimmune attack seen in vitiligo results in depigmented skin. Despite a common autoimmune target, the melanocyte, these two diseases are infrequently co-expressed and rarely co-localised. As such, we hypothesized that there must be fundamental antigenic differences between melanocytes of the skin and those of the hair follicle. In order to examine ex-vivo expression of MHC antigens on melanocytes of the hair follicle and epidermis, we performed double immunofluorescent staining on scalp cryosections from normal subjects with either w6/32 or CR3/43 and NKI/beteb recognizing MHC-Ia or MHC-II expression and melanocytes respectively.

We found that anagen hair follicle melanocytes residing above the apex of the dermal papilla do not express MHC-Ia or MHC-II. Epidermal melanocytes however, do express MHC-II. The expression of MHC-Ia on epidermal melanocytes could not be conclusively determined. Due to the strong staining with w6/32 by epidermal keratinocytes, NKI/beteb-labeled melanocytes could not be accurately identified through double-staining. Our finding of a contrasting MHC expression pattern between these anatomically distinct melanocyte populations may further our understanding why hair follicle melanocytes are preferentially attacked in alopecia areata whereas epidermal melanocytes are the target in vitiligo.

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A Characterisation of *i*NKT Cells in Mouse Skin

Kirsten Hammond, Carling Chan and Gary Halliday *Dept of Dermatology, Sydney University*

'Invariant' (*i*) NKT cells are an evolutionarily conserved T cell subset that use a single T cell receptor (TCR) α chain (V α14J α18 in mice; V α24J α18 in humans) combined with a limited repertoire of TCR *β* chains. The highly restricted TCR repertoire of *i*NKT cells recognises glycolipid antigens presented by the antigen-presenting molecule CD1d, which immediately distinguishes them from most other α *β* T cells that recognise peptides in conjunction with major histocompatibility complex (MHC) class I or II molecules. *i*NKT cells have the ability to powerfully influence immune responses due to their capacity to rapidly produce copious amounts of immuno-modulatory cytokines.

Here we report for the first time the direct identification of *i*NKT cells isolated from normal mouse skin. We used a highly specific reagent, αGalCer-loaded CD1d tetramers, and flow cytometry to detect these cells. This observation has important implications for skin immunity – for example, a glycolipid extracted from *Borrelia burgdorferi*, the causative agent of Lyme disease which infects humans through deer tick bites, has recently been found to stimulate *i*NKT cells. In addition, *i*NKT cells have been implicated in the mechanism by which ultraviolet (UV) radiation from sunlight suppresses the immune system – a significant contributing factor in the development of skin cancer induced by UV radiation. Our novel findings, that *i*NKT cells are present in skin in large numbers, indicate that these cells may represent major mediators of skin immunity.

Investigating the Role of UV-Activated Cell Signalling Pathways in TNFα released from Keratinocytes

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Background: UV exposure causes direct DNA damage resulting in multiple mutations as well as suppressing the body's immune system. Mutated genes have a role in pro-tumour activities that can lead to the development of skin cancer. However, before UV radiation on gene expression can be elicited, signalling pathways first need to be activated. There are three major signalling pathways in the cell which are activated by UV radiation; p38 MAPK, JNK and NF-κB pathways. It is unknown as to which pathway plays a major role in UV-induced TNFα release from skin cells.
Method: The human keratinocyte cell line, HaCaT, and squamous cell carcinoma cell line, Colo16 were exposed to UV (A, B and combinations thereof) radiation and changes in the cellular expression of p38 MAPK, JNK and NF-κB pathway intermediates over a 24 h period were measured using Western blots. TNFα levels in the culture media were measured using ELISA kits.
Results: Cell cultures were exposed to either UVA (2000 and 20000 Jm^{-2}) and/or UVB (200 and 2000 Jm^{-2}) radiation. Colo 16 cells were more sensitive to high dose UVB and UVA + B radiation than were HaCaT cells, though neither cell line was sensitive to UVA radiation at the doses tested. Of interest was the finding that in both HaCaT and Colo 16 cells, the increase and decrease of IêBá levels which indicated the inactivation and activation of NFêB was observed at different time points over the 24 h time period.
Conclusion: The results obtained from these studies, show that UV radiation activates different responses in HaCaT and Colo 16 cells. In both cell lines, UVB and UVA + B radiation appear to be more detrimental than does UVA. While HaCaT cells are used by many as a model for keratinocyte biology, their role in cell signalling studies needs to be re-evaluated as they contain deficiencies in some signalling and cell cycle intermediates [1].

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Abstracts

Comparison of the Immune Suppressive Effectiveness of UVB and Long-wave UVA in Humans

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Background: Ultraviolet radiation (UVR)-induced suppression of cutaneous immune responses enhances the development of skin cancers. We determined the immune suppressive effectiveness of various narrow bandwidths across the UVR spectrum in order to calculate the relative immunosuppressive contribution of UVB (290–320 nm) and UVA (320–400 nm) in sunlight.
Method: We recruited groups of healthy, nickel-allergic female volunteers. Using a filtered xenon arc lamp, we exposed discrete areas of skin on the lower back of volunteers to graded doses of narrowband UVR. Adjacent, unirradiated areas of skin served as immunologically intact control sites. Following UVR exposure, nickel patches were placed on each of the irradiated and unirradiated sites. The intensity of nickel contact hypersensitivity responses at each site was then measured with a reflectance erythema meter as nickel-induced erythema (NIE) 72 hours later. The degree of immunosuppression was calculated by comparison of the NIE of irradiated sites to the NIE of control unirradiated sites.
Results: The UVB wavelengths 290–310 nm caused significant dose-responsive immunosuppression at suberythemal doses. The UVA wavelengths 360–380 nm were also immunosuppressive at exposures equivalent to only 15 minutes of Spring midday sun in Sydney, with loss of the effect at higher exposures.
Conclusion: There are two peaks in the action spectrum for immunosuppression in humans with fundamental differences in the dose-response characteristics between these peaks. UVA is approximately 20 times more abundant in solar radiation than UVB and therefore contributes relatively more to the immunosuppression that occurs following brief exposures.

Unlocking the Inductive and Stem Cell Potential of Hair Follicle Mesenchyme

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The hair follicle dermis/mesenchyme has well-established inductive potential in which skin epidermis can be induced to form hair follicles. There is also evidence that under their influence other epithelia, including corneal epithelium from the adult eye, can be reprogrammed into skin and follicles. Dermal stem cell activity has been recognized for some time in the context of experimental follicle regeneration and wound healing, however more recently a much broader stem cell potential has emerged. Follicle dermal cells are also able to support other stem cells in an undifferentiated state. These findings have raised interesting therapeutic possibilities, as well as some intriguing biological questions concerning the relationship between induction and stem cell activitie

Recessive Epidermolysis Bullosa Simplex due to a Homozygous Truncation Mutation KRT14 Y204X with Basal K6/16 Expression and an Ameliorating Phenotype

Linda Martin[1], Eleni Yiasemides[1], Niken Trisnowati[1,4], John Su[2], Sandra Klingberg[3], Penelope Marr[4], David Orchard[2], George Varigos[2] and Dédée F Murrell[1,5] *[1]Department of Dermatology, St George Hospital, Sydney; [2]Royal Children's Hospital, Melbourne; [3]Chemistry Laboratory, Royal Brisbane Hospital; [4]Dept of Anatomical Pathology, St George Hospital; [5]The University of NSW, Sydney*

Background: Epidermolysis bullosa simplex (EBS) is usually inherited as an autosomal dominant trait due to mutations in either *KRT5* or *KRT14*. Recessive EBS is extremely rare and only 6 previous mutations in *KRT14* have been described, and others in *PLEC1*. Here we present the first Australian patient diagnosed with recessive EBS due to KRT14 Y204X and compare the phenotype with the previous cases of R-EBS.
Case Report: A female child of consanguineous parentage was noted at birth to have aplasia cutis affecting the palms and soles. Blistering worsened and she had failure to thrive and anaemia when very young which gradually improved with age. At 8 years she had scattered blisters, mucosal involvement, palmoplantar hyperkeratosis, hyperkeratotic nails, pruritus, epidermolysis bullosa (EB) naevi and atrophic scarring.
Results: Electron microscopy demonstrated blistering in the basal layer consistent with epidermolysis bullosa simplex (EBS). Keratin tonofilaments were absent in the basal layer, however normal keratin filaments were observed in the stratum spinosum and above. Immunofluorescence mapping was negative for K14, and, unusually, was positive for K6 and 16. Mutation screening identified a homozygous truncation mutation, *KRT14*-Y204X.
Conclusion: Keratins 5 and 14 are obligate heterodimers and interact to form the intermediate filament cytoskeleton, which is essential for maintaining the integrity of the basal epidermis. The *KRT14*-Y204X mutation is predicted to destabilise mRNA, resulting in nonsense-mediated decay of K14. The proband's phenotype was relatively mild considering that no K14 protein was expressed in her skin. Keratin 6 and 16, which are generally only present in hyperproliferative disorders were expressed in her epidermis. It is proposed that expression of these keratins compensates for the loss of K14, and attenuates disease phenotype.

Novel and Recurrent Mutations in KRT5 and KRT14 in Epidermolysis Bullosa Simplex in Australia

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Background: Epidermolysis bullosa simplex (EBS) is a group of heritable blistering skin disorders caused mostly by mutations in the keratin genes KRT5 and KRT14. EBS has been divided into four subtypes according to the clinical severity and distribution of the lesions.
Methods: EBS was clinically diagnosed and confirmed by transmission electron microscopy and immunofluorescence mapping examination of a skin biopsy. Genomic DNA was extracted from peripheral blood leucocytes and mutation analysis of KRT5 and KRT14 was performed by direct sequencing in Australian patients.
Results: We have diagnosed 32 EBS families and screened 22 of these for KRT5 and KRT14 mutations. 8 mutations were identified in KRT14 and 4 in KRT5 and 2 mutations in KRT5 combined with KRT14. 11/22 were sporadic cases. In 8 cases, no mutations were found. 7 mutations had not been previously published when identified, including KRT14 M119T, M119V, M272T and KRT5 E168D, 429delRNKLA, D197E, K199N.I n addition, we got 13 cases with autosomal dominant inheritance, and 1 case with recessive type. Thus far, none have been used for pre-natal diagnosis.
Conclusions: All mutations with severe clinical manifestations (clinically classified as EBS-DM) cluster in the highly conserved ends of the alpha-helical rod domain (HIP,HTP). However, not all mutations present in hotspots result in EBS-DM. Even different amino acid substitutions at the same locus and the same mutation can also lead to different clinical severities. Moreover, most of our patients are improving with age, even in EBS-DM cases. This suggests that there are some disease-modifying genes in EBS or other protein interactions which play an important role. This area requires further research as manipulation of these genes could potentially ameliorate the phenotype earlier.

Review of Collagen VII Mutations Found in Australian Patients with Dystrophic Epidermolysis Bullosa Reveals 9 Novel COL7A1 Mutations

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Introduction: Dystrophic Epidermolysis bullosa (DEB) is an inherited skin fragility disorder whereby blistering occurs in the sub-lamina densa zone at the level of anchoring fibrils (AFs) of the dermo-epidermal junction1. It is inherited in both an autosomal dominant (DDEB) and an autosomal recessive manner (RDEB). Both RDEB and DDEB result from mutations in the type VII collagen gene (COL7A1).
Method: Skin biopsies from patients were processed for IF antigen mapping. Where there were sub-lamina densa splits and/or a reduction or negative Collagen VII antibody staining, the COL7A1 genes were screened for mutations. A review of known mutations of the type VII collagen gene and the genotypes and phenotypes of the patients with DEB was undertaken.
Results: We report 15 Australian families with different forms of dystrophic epidermolysis bullosa (DEB) with 23 different COL7A1 allelic mutations, 9 of which were novel mutations. 4 cases of RDEB-HS combined two PTC mutations and 3 cases of RDEB-HS combined a PTC in one allele with a second spice-site or silent glycine substitution mutation in the other allele. G2043R, a *de novo* and dominant mutation, was identified again in this study. Four recessive silent glycine substitution mutations were found, G2775S and G1673R (novel), G1338V (novel) and G2791A. Family 3 with DDEB combined R27 91W and novel G2210V and had a Pasini phenotype in most individuals, but two members of the family had severe DDEB pruriginosa. From these 3 prenatal diagnoses have been performed.

References

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Acknowledgement

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Is the Mathematical Modelling of Skin Biology of any Use?

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Although mathematics now occupies centre stage in many aspects of biological investigation – think of the rise in importance of data analysis in bioinformatics – there remain within the scientific community reservations about the practical utility of mathematical models in the life sciences. Here I aim to show, by counter – example, how these reservations are unfounded. I will first demonstrate how quantitative models can make correct biological predictions, and then describe how the results of experiments guided by prediction can validate the postulated biological principles incorporated a *priori* into the models. Finally, I will argue that the process of modelling coupled to experiment and observation in an *iterative* fashion will, by necessity, lead to a paradigm shift in the investigation of skin disease.

Analysis of the Estrogen Receptor Beta Gene, *ESR2*, In Female Pattern Hair Loss

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Background: Female pattern hair loss (FPHL) has a polygenic mode of inheritance with an unproven relationship with androgens. The genes involved in its pathogenesis remain unknown, but are likely to include genes related to the androgen and estrogen pathways. The *ESR2* gene is located on chromosome 14q22-24 and codes for the estrogen receptor beta (ER-â). In contrast to ER-á, ER-â is the predominant subtype within the outer root sheath and epithelial matrix, making it likely that the *ESR2* gene directly modulates the hair growth cycle. This study aims to evaluate the relationship between *ESR2* gene variants and FPHL.
Method: Allele and genotype frequencies of tag single nucleotide polymorphisms (tag SNPs) in the *ESR2* gene were compared between 150 cases with FPHL (stage 3 or greater) and 90 controls (aged >50 years with no hair loss conditions), using χ^2-tests. Tag SNPs are representative variants in a region of linkage disequilibrium, whereby their examination would be sufficient to capture the genotypic information of all known SNPs in that region.
Results: Three *ESR2* tag SNPs that collectively capture 33 SNPs in the gene have been analysed and there were no significant differences in allele or genotype frequency between cases and controls ($P>0.1$).
Conclusion: By essentially examining 33 SNPs located in the *ESR2* gene region, we have been unable to identify genetic association between *ESR2* and FPHL. However, the association of this gene with FPHL cannot yet be discounted. We are currently analyzing the remaining tag SNPs necessary to capture all known SNPs in the gene region, and are recruiting an additional 275 cases and 400 controls through the Melbourne Collaborative Cohort Study to increase statistical power of this study.

POSTER PRESENTATIONS

Donor site dominance in action: transplanted hairs retain their original hair pigmentation long-term.

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The concept of 'donor dominance' in hair transplantation refers to autografts which continue to maintain their integrity and characteristics after transplantation to a new site1. Such hairs may retain their original texture and rate of growth. Hair transplantation for patients with androgenetic alopecia rely on this concept of donor dominance for a successful and long-lasting result. Recently, the concept of 'recipient dominance' in hair transplantation has been debated. In a study of patterns after hair transplantation to the scalp and eyebrows in patients affected by madarosis, Lee et al2 found that the greying rate of hairs approximated the recipient site rather than the donor site.
We report on the long-term maintenance of follicular pigmentation in transplanted hairs. We describe two patients affected by both androgenetic alopecia and hair greying in the transplant recipient area. They were given autografts of normally pigmented hair follicles harvested from the occipital area. More than one year post-transplantation, their donor hairs have remained pigmented long-term, despite being implanted in scalp affected by greying. In one patient the pigmented hairs have remained stable for 10 years. As the process of greying usually affects the temporal scalp first, then progresses onto the vertex and occiput later, the maintenance of long-term follicular pigmentation in our patients may be attributable to donor dominance.

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A Ten-Year Review of Skin Cancer in 588 HIV-Positive Patients

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Background: Our aim is to review the types and prevalence of skin cancer among HIV-positive patients presenting to two dermatology clinics, and identify the association between sun related skin conditions, skin cancers seen, age, CD4 count and duration of HIV infection.
Methods: This retrospective study reviews 588 HIV-positive patients who attended a dermatologist's clinic at the Skin and Cancer Foundation in Darlinghurst, and her private dermatology office in St. Leonard's, Sydney Australia. Information obtained for this study included: age, sex, duration of HIV-infection, CD4 count and viral load at time of first visit, AIDS status, current treatment and main diagnoses.
Results: 191 (32.48%) patients had one or more skin malignancies, of whom 157(26.70%) had a sun related skin cancer (SRSC). These included 13 (22.62%), with one or more basal cell skin cancers (BCC), 48 (8.16%) who had invasive or in-situ SCC, and 7 (1.19%)who had a melanoma...Actinic keratoses were diagnosed in 149 out of 588 patients (25.34%).. The ratio of patients with BCC to patients with SCC was 2.77:1, Non-sun related cancers included 31(5.27%) with KS and 15(2.55%) with anal dysplasia (including AIN and Bowenoid papulosis).
Conclusion: This study highlights the importance of monitoring for SRSC among HIV-patients. SRSC were associated with an age greater than 40-years and mild HIV-immunosuppression (CD4 count >450 cells/µl). An association between BCC and duration of HIV-infection was identified (P=0.032, 95%CI: 0.113 to 2.518), but the confounding factor of age makes this difficult to interpret. KS and anal dysplasia were associated with moderate immune deficiency (CD4 count between 250-350 cells/µl).

Differential expression of pyloric atresia in junctional epidermolysis bullosa with novel ITGB4 mutations

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Background: Junctional epidermolysis bullosa with pyloric atresia is an autosomal recessive blistering disease including lethal and non lethal variants due to mutations in ITGB4 and ITGA6.
Method: Skin biopsies from patients were processed for IF mapping and when staining for integrin beta 4 or alpha 6 were absent or reduced, *ITGB4* was screened for mutations. A review of known mutations of *ITGB4* and the phenotypes of the patients with JEB-PA was undertaken.
Results: 3 novel ITGB4 mutations were identified in 3 families with JEB -PA: two splice-site and one insertion mutation. Families 1 and 2 with lethal phenotypes were due to combinations of PTC and missense mutations (658delC/R252C and 3903dupC/G273D, respectively). However, two cases in our report had no gastrointestinal symptoms or signs of pyloric atresia (PA). (1) Family 2, infant born at 33/40 weeks, with marked aplasia cutis, especially around the neck, prominent subcutaneous veins, and dysmorphic facies, who died after 1 day; (2) Family 3, an affected sister and brother, had only mild skin involvement with blistering on the feet in summertime. Although both were homozygous for *ITGB4* 264G-A/3111-1G-A, only the brother had PA.
Conclusion: These results suggest that pyloric atresia is an inconstant feature of the subtype of EB known as JEB -PA and that another factor must be determining whether the patient presents with PA or not. It could be that institutions which do not routinely screen IFM for integrin a6b4 staining in the absence of PA could be missing this form of EB.

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Randomised, double-blind, prospective study to compare topical 5-aminolaevulinic acid methylester with topical 5-aminolaevulinic acid photodynamic therapy for extensive scalp actinic keratosis.

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Background: 5-aminolaevlinic acid methylester (MAL) and 5-aminolaevulinic acid (ALA) photodynamic therapy (PDT) are both effective treatment options for actinic keratosis (AK)s. While MAL is significantly more expensive than ALA, no studies have directly compared their efficacy in the treatment of extensive scalp AKs.
Methods: To compare the efficacy and adverse effects of MAL-PDT with ALA-PDT in the treatment of scalp AKs, 16 male patients aged 59 to 87 with extensive scalp AKs were randomized into a double-blind, split-scalp prospective study. Two treatment fields were defined (right and left frontoparietal scalp) and treated two weeks apart. These fields were randomised to receive either MAL or ALA as first or second treatment. MAL cream was applied for three hours. Twenty per cent ALA cream was applied for five hours. A blinded observer assessed efficacy comparing AK counts before and one month after treatment. Pain was assessed using a visual analogue scale at 3, 6, 12 and 16 minutes.
Results: 15 patients completed treatment to both fields. There was a mean reduction from baseline in AK counts with the use of ALA of 6.2+/-1.9 as compared to 5.6+/-3.2 with MAL-PDT (p=0.588). All patients experienced pain which was of greater intensity in the ALA treated side at all time points: 3 minutes p=0.151, 6 minutes p=0.085, 12 minutes p=0.012 and 16 minutes p=0.029. Similarly, duration of discomfort post procedure persisted for longer following treatment with ALA when compared with MAL-PDT (p=0.044)
Conclusion: This study demonstrates that both ALA and MAL-PDT result in a significant reduction in scalp AKs. There is no significant difference in efficacy. However, ALA is more painful than MAL-PDT in the treatment of extensive scalp AKs.

Subcutaneous Injections: What are we doing to our skin?

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Background: Nearly 150 years have passed since the first documented use of subcutaneous injections was described for mercury compounds to evade the scourge of syphilis. Since this time, numerous agents for subcutaneous injection have been developed for medicinal, recreational and cosmetic purposes. Currently, over 66 drugs are available for medicinal use as subcutaneous injections in Australia and all have been associated with local skin reactions. Some of these adverse events are well recognised including: insulin (lipoatrophy), vaccines (pain and swelling), vitamin K (localised sclerodermoid plaques), silicon (siliconomers) and heparin (localised skin necrosis).
An increasing number of novel biological agents are now being injected subcutaneously as they are not tolerated orally. These include: interleukin-2 (for the treatment of HIV and numerous metastatic cancers); beta?interferon (for the treatment of multiple sclerosis and hepatitis C); etanercept (for the treatment of psoriatic arthritis, psoriasis and rheumatoid arthritis); glatiramer acetate (for the treatment of multiple sclerosis) and enfuvirtide (an antiretroviral agent for HIV-1 infection).
Case Report: We report four cases in which subcutaneous injections have produced panniculitis with and without necrosis. These have resulted in atrophy and/or sclerosis caused by very different physiologic pathways, producing diverse histopathological pictures. These cases include reactions to beta?interferon, interleukin, enfuvirtide and glatiramer acetate. The significant reactions described are considerably worse than the reported mild and well tolerated cutaneous reactions reported by the manufacturers.

Results from a study on the role of *Staphylococcus epidermidis* in the pustules of acne rosacea.

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Background: Rosacea is a common skin disease characterised by facial flushing, telangiectasia, papules and pustules. It is generally regarded as inflammatory in nature. We believe that a contributory role of bacteria in this condition needs to be revisited, particularly in the light of the recognition of the role of *Propionibacterium acnes* in acne vulgaris and the use of topical and systemic antibiotics as a mainstay in the treatment of rosacea.
Aims: (1) To ascertain whether there is a difference in the bacteria present on the facial skin of patients with rosacea and those without. (2) To ascertain whether there is a contributory role of bacteria in the formation of pustules seen in acne rosacea.
Methods: This was a two-party study; the first part being a case-controlled trial with clinically and historically diagnosed pustular rosacea patients. Bacteria were isolated from the patient's pustule, and in addition swabs were taken from the inferior eyelid margin and the facial skin near the pustule that was incised. This was then compared to swabs taken from controls. The second part, a cross-sectional analysis, involved comparing the type of bacteria isolated from the patient's pustule to the type of bacteria isolated from the skin swab of the same case. Specimens were cultured aerobically and anaerobically. Speciation as well as antibiotic sensitivity of all pure growths of staphylococci isolates was performed.
Results: (1) There was no significant difference in the type of bacteria isolated from the skin of cases and skin of controls (*Micrococcus*: p = 0.143, *Diphtheroids*: p = 0.136, *Staphylococcus*: p = 0.439 and *P.acnes*: p = 0.273). (2) There was a statistically significant difference in the type of bacteria isolated from the pustule of cases compared with the bacteria isolated from the skin of the same case. Nine out of fifteen pustule lesions cultured pure growth of *Staphylococcus epidermidis*, whereas all fifteen-skin swabs cultured mixed growths (p = 0.001).
Conclusion: Our findings strongly implicate *Staphylococcal epidermidis* in the formation of the pustules seen in acne rosacea. The long history of flushing prior to the appearance of telangiectasia and pustules, points to the possibility that the persistent flushing produces an altered environment, which may encourage the growth of Staphylococcus epidermidis and its subsequent formation of pustules.

Long-term hair repigmentation following a hair transplant for frontal scarring alopecia

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Hair greying invariably manifests as part of ageing and is considered irreversible. Pigment loss in canities is due to a reduction or absence of actively melanogenic hair follicle melanocytes in grey and white hairs respectively. There is also dysfunctional pigment transfer to cortical keratinocytes as a result of melanocyte degeneration. Amelanotic melanocytes of the mid-to-lower outer root sheath (including the bulge region) are still present in white hairs but incomplete melanocyte stem cell maintenance leads to greying[1]. Repigmentation of grey or white hairs can occur with certain medical conditions[2], usually as a post inflammatory phenomenon, and as a side effect of some medications. However, most cases of repigmentation are unsustained.
We describe pronounced and long-term repigmentation of white donor hairs in a 57 year old woman who underwent hair transplant surgery for an area of frontal scarring alopecia secondary to a burn from childhood. The repigmented hairs have remained stable at more than two years post transplant. Although repigmentation of hairs is thought to be explained as possibly a post-inflammatory phenomenon or secondary to altered signally in the hair bulb pigmentary apparatus after surgery, the mechanisms behind the long-term upkeep of the repigmentation remain elusive. Further studies focussing on the pathophysiology of the repigmentation mechanisms will advance the understanding of the greying process. Such studies may also provide insights into strategies to reverse hair greying in the future.

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